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The use of rat spinal reflexes to quantify injection pain

Nozomi Ami *, Hideki Satou

R&D Center, Terumo Co., 1500 Inokuchi, Nakai-machi, Ashigarakami-gun, Kanagawa 259-0151, Japan

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ABSTRACT

Pain caused by subcutaneous injections is unpleasant, which may limit patient compliance. The objective of this study was to use spinal reflexes to quantify subcutaneous injection pain. Spinal reflexes were measured using an electromyogram (EMG) test. The effects of injection volume, pH and osmotic pressure were investigated. The EMG responses increased with injection volume and the acidity of the solution but did not depend on the osmotic pressure of the solution. The EMG responses differed for subcutaneously injected sodium chloride and glucose over the same range of osmotic pressures. Pain caused by the subcutaneous injections was unrelated to the osmotic ratio up to approximately 5. The injection pain caused by therapeutic protein solutions was also evaluated. We compared the EMG responses of the adalimumab and etanercept, as the injection of adalimumab is more painful than that of etanercept in humans. The EMG magnitude for adalimumab was twice that induced by etanercept as observed for the EMG tests performed in rats. Therapeutic proteins account for an increasingly large proportion of pharmaceutical drugs. When a high dose of therapeutic proteins is required, the protein solution must often be highly concentrated to reduce the injection volume. For patient comfort, it is critical to reduce injection pain. The EMG test reported here allows subcutaneous injection pain to be quantified and may be useful for optimizing drug formulations.

1. Introduction

Previous studies have shown that the assessment of the spinal flexor reflex evoked by noxious stimuli in anesthetized rats is a useful method for assessing nociceptive pain caused by needle insertion (Okamoto et al., 2012). In this study, we aimed to apply this method to quantify subcutaneous drug injection pain in animals. We injected various formulations into rat plantar aspect subcutaneously and measured the corresponding muscle contractions using electromyogram (EMG) to assess pain. For example, if one steps on a pushpin, one withdraws their leg from the pushpin. When the leg is withdrawn, leg muscles contract, and the degree of the contraction depends on the intensity of the stimulus. Based on this phenomenon, we believe that subcutaneous injection pain can be measured using EMG.

The pain induced due to a subcutaneous injection depends on several factors, including the pH and the osmolarity of the solution (Jørgensen et al., 1996) and the buffer (Laursen et al., 2006). We sought to quantify injection pain and to investigate several factors that influence EMG responses.

2. Material and methods

2.1. Animals

Male Crl:CD (SD) rats (Charles River Laboratories Japan, Yokohama, Japan), aged 8 weeks, were used in this study. There were 8 animals in each study group. The animals were housed 2 per cage under controlled temperature and humidity (23 ± 2 °C, 50 ± 10%) and on a 12-h light/dark cycle. The rats had access to tap water and food *ad libitum*. This study was approved by the Committee for Animal Experiments at the Terumo Corporation, and care and use of the animals conformed to the applicable guidelines of the Science Council of Japan.

2.2. Measurement of electromyograms (EMG)

The rats were anesthetized with 3% isoflurane (Escain®, Pfizer Japan, Tokyo, Japan), and a superficial incision was made in the skin of the hindlimb. A bipolar stainless steel electrode (TH209-024, Unique Medical, Tokyo, Japan) was inserted into the

Abbreviations: EMG, electromyogram; PBS, phosphate-buffered saline; SEM, standard error of the mean.

* Corresponding author. Tel.: +81 465 81 4157; fax: +81 465 81 5372.
E-mail address: Nozomi_Ami@terumo.co.jp (N. Ami).

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semitendinosus muscle to a depth of 2 mm. The inter-electrode distance was 1 mm, and a length of the exposed tip was 0.2 mm. The EMG signals were amplified with a gain of 20,000 and filtered (high pass filter at 100 Hz and low pass filter at 3000 Hz) using an extracellular amplifier (ER-1, Cygnus Technology, Delaware Water Gap, PA, USA) (Chang et al., 2007). The EMG signals were recorded using PowerLab® (input impedance; 1 MΩ at 100 pF, CMRR; 100 dB at 100 Hz, 16 bit A/D conversion, AD Instruments, Dunedin, New Zealand) and LabChart® (AD Instruments) with a sampling rate of 10 kHz/s.

A clip-type electrode (TH207-123, Unique Medical) was applied to the hind paw, and an electrical stimulus (2 ms, 5 mA, 40 Hz) was delivered using a biphasic stimulus isolator (BSI-950, Dagan, Minneapolis, MS, USA). The anesthesia level was adjusted until three consecutive suitable EMG response (amplitude > 100 μV, duration of response > 10 s) were obtained. The electrical stimuli were given at 10 min intervals. The anesthesia was adjusted to 1–1.4%.

The area of the full wave rectified form within the EMG signals was integrated to yield the magnitude of the EMG response.

2.3. Injection

Test solutions were injected into the rat plantar aspect subcutaneously using a syringe pump (MCIP-III, Minato Concept, Tokyo, Japan). The injection speed was 10 μL/s. For injection, a 1 mL syringe (SS-01T, Terumo, Tokyo, Japan) was used. A 27 gauge blunt needle (Instech Laboratories, Plymouth Meeting, PA, USA) and a polyurethane tube (Micro-renathane® 025, Braintree Scientific, Braintree, MA, USA) were attached to the syringe, and an injection needle (29G, Terumo) was attached to the other end of the injection tube. The injection needles were inserted subcutaneously. Test solutions were injected when an EMG response evoked due to the needle insertion was observed. The injection needles were retracted when the EMG responses were terminated. Test solutions were injected at 10 min intervals, and the injection sites were more than 2 mm apart. The injection order randomized for each animal.

2.4. Injection solutions

To evaluate the injection volume, saline (Otsuka Normal Saline, Otsuka, Tokyo, Japan) was injected at a volumes of 20, 50 and 100 μL.

To evaluate pH, PBS (0.01 M, Invitrogen, Carlsbad, CA, USA) was used as a basic solution. The pH of each stimulus solution was measured using a pH meter (D-51, Horiba, Kyoto, Japan) and adjusted with 1 N HCl to pH values of 7, 6, 5, 4 and 3. Each solution was injected at a volume of 20 μL.

To evaluate the osmotic pressure, a 50% glucose solution (Otsuka) and a 10% sodium chloride solution (Otsuka) were used. The glucose solution was diluted with distilled water to 5%, 10% and 20%. The sodium chloride solution was diluted with distilled water to 0.9%, 2% and 5%. The pH of each solution was adjusted to approximately neutral. Twenty microliters of each solution were injected. The osmotic pressure of the solution was measured using an osmometer (Advanced® 3D3, Advanced Instruments, Norwood, MA, USA).

To evaluate the biological medicines, adalimumab (50 mg/mL) and etanercept (50 mg/mL) were injected twice in each animal. The drugs were allowed to equilibrate to room temperature for at least 30 min. The injection volume was 20 μL.

2.5. Statistical analysis

The results are expressed as the mean ± standard error of the mean (SEM). The data were analyzed using a one-way analysis of variance followed by Tukey's *post hoc* test. For biological medicines, an unpaired *t*-test was performed. Values of $P < 0.05$ were considered statistically significant.

3. Results

3.1. Experimental procedure and typical EMG response

Fig. 1A shows the experimental procedure.

Fig. 1B shows a representative EMG response. The arrows indicate the time points of needle insertion and solution injection.

3.2. Injection volume

The EMG magnitude increased with increasing injection volumes. The EMG magnitudes for injections of 20, 50 and 100 μL were 43.3 ± 21.6, 375.2 ± 139.6 and 473.5 ± 117.0 μV · s, respectively. The difference in EMG magnitude between 20 and 100 μL injections was significant (Fig. 2).

3.3. pH

The EMG magnitudes elicited due to injections of solutions with pH values of 7, 6, 5, 4 and 3 were 43.4 ± 28.4, 169.1 ± 43.5, 226.6 ± 48.4, 235.5 ± 62.4 and 346.0 ± 98.8 μV · s, respectively (Fig. 3), and a significant difference was observed between pH 7 and pH 3.

3.4. Osmotic pressure

The osmotic pressures for 0.9%, 2%, 5% and 10% sodium chloride were 292, 633, 1583 and 3282 mOsm/kg H_2O, respectively. The EMG magnitudes elicited due to these solutions were 124.8 ± 87.4, 198.6 ± 143.6, 529.0 ± 93.7 and 851.8 ± 184.1 μV · s, respectively (Fig. 4A). Significant differences were observed between 0.9% and 10% and between 2% and 10%.

The osmotic pressures for 5%, 10%, 20% and 50% glucose were 292, 610, 1347, and 2917 mOsm/kg H_2O, respectively. The EMG responses were very small for the doses of 5%, 10% and 20%. The EMG response elicited due to the 50% solution was 345.9 ± 111.6 μV · s (Fig. 4B).

3.5. Biologic medicines

The EMG magnitudes elicited due to administration of adalimumab and etanercept were 346.1 ± 87.2 and 147.6 ± 44.9 μV · s

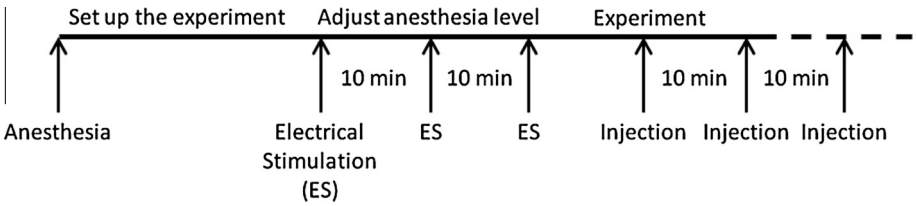

Fig. 1A. The experimental procedure.